Statistical Optimization of Seed Coating in Arid-Zone Grass Species Used as Cover Crops in Woody Agroecosystems

Optimización estadística del recubrimiento de semillas en gramíneas de zonas áridas para cultivos de cobertura en agroecosistemas leñosos

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Abstract

Inter-row vegetative cover improves soil physicochemical properties and biodiversity but should not compete with main crop for water. Native grass species such as *Leptochloa crinita* (Lag.) P. M. Peterson & N. W. Snow, *Pappophorum caespitosum* R. E. Fr. and *Digitaria californica* (Benth.) Henrard var. californica provide forage value, require less water than conventional cover crops, and exhibit high tolerance to the edaphoclimatic conditions of arid regions. However, their small seed size hinders efficient handling and sowing. Seed coating increases seed size and weight, forming a pellet that improves handling, distribution, and protection. Nevertheless, limited information is available on coating techniques for small-seeded species. This study aims to optimize the coating process through a statistically designed experiment to evaluate key morphological and physiological seed traits. A Box-Behnken design was applied to optimize one compositional factor of the pellet and two operational parameters. Pellet traits such as size, heterogeneity, seed number, and residue were analyzed alongside germination and seedling development. Optimization was first carried out using edible amaranth seeds as a model organism and then validated with native grasses. The resulting models were statistically significant and allowed the identification of an optimal operational range. Germination was unaffected in single-seed pellets, and root growth was enhanced by the coating process. Overall, the proposed statistical methodology proved effective and broadly applicable for optimizing small-seed coating processes.

Keywords

Native seeds • *Leptochloa crinita* • *Pappophorum caespitosum* • *Digitaria californica*

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Resumen

Las coberturas vegetales interfilares mejoran las propiedades fisicoquímicas y la biodiversidad del suelo, pero no deberían competir por el agua en los cultivos. Algunas especies nativas, como Leptochloa crinita (Lag.) P. M. Peterson & N. W. Snow, *Pappophorum caespitosum* R. E. Fr. y *Digitaria californica* (Benth.) Henrard var. californica, ofrecen valor forrajero, requieren menos agua que cultivos de cobertura convencionales y presentan alta tolerancia a las condiciones edafoclimáticas del árido. No obstante, el pequeño tamaño de sus semillas dificulta su manejo. El recubrimiento incrementa tamaño y peso de las mismas, formando un pellet que facilita su manipulación, distribución y protección. Sin embargo, hay escasa información sobre el recubrimiento de semillas pequeñas. Este estudio busca optimizar dicho proceso mediante diseño experimental y evaluar variables morfológicas y fisiológicas clave. Se aplicó un diseño Box-Behnken para optimizar un factor de composición del pellet y dos factores operativos. Se evaluaron características del pellet como tamaño, heterogeneidad, cantidad de semillas y residuo, así como la germinación y desarrollo de plántulas. La optimización se realizó con semillas de amaranto de consumo humano como organismo modelo y fue validada en gramíneas nativas. Los modelos resultaron significativos y permitieron definir una zona operativa óptima. La germinación no se vio afectada en pellets de una semilla, más aún el crecimiento radicular se vio beneficiado por el proceso de recubrimiento. Finalmente, la metodología estadística utilizada demostró ser aplicable y eficaz para optimizar el recubrimiento de semillas.

Palabras clave

Semillas nativas • *Leptochloa crinita* • *Pappophorum caespitosum* • *Digitaria californica*

Introduction

Some agronomic management practices in woody crops like vineyards, olive groves, almond orchards, and walnut plantations can act as regionally significant drivers of soil degradation. Inter-row areas in these systems, particularly under pressurized irrigation, are often classified as "degraded areas" due to prolonged soil exposure to erosive forces (Chen *et al*. 2010).

Inter-row cover crops provide multiple agro-environmental benefits reducing weed competition, enhancing biodiversity, improving nutrient cycling, and serving as green manure (Rubio-Asensio *et al.* 2022; Thioye *et al.* 2021; Tomaz *et al.* 2021). However, in semi-arid regions, conventional cover crops may compete with main crop for water and nutrients. This limitation encourages the use of native species, which generally possess deep root systems, lower water requirements, and superior adaptation to local edaphoclimatic conditions (Pornaro *et al*. 2022; Massa Decon & Pérez, 2025).

Native grasses such as *Leptochloa crinita* (Lag.) P. M. Peterson & N. W. Snow, *Pappophorum caespitosum* R. E. Fr. and *Digitaria californica* (Benth.) Henrard var. californica are valued for their tolerance to drought, aridity and salinity, rapid establishment, and low water use (Kozub *et al*. 2018; Vega-Riveros *et al*. 2020). *D. californica* has already been used successfully as an inter-row species (Ferrari & Parera, 2015), while *L. crinita* and *P. caespitosum* have been employed as forage grasses (Marinoni *et al*. 2019). Nevertheless, these native species produce small, lightweight seeds (often less than 1 mm), with variable shapes (Carballo *et al*. 2005; Ramírez-Segura *et al*. 2022), making them difficult to handle and sow (Pedrini *et al*. 2017). Germination rates also improve significantly when floral residues are removed, exposing caryopsis (Meglioli *et al*. 2024).

Seed coating intends to overcome these challenges. By enclosing the caryopsis within a protective layer, coating increases seed size, enhances mechanical and distribution, and reduces damage during sowing (Afzal *et al*. 2020; Pedrini *et al*. 2017). Moreover, it can improve germination and plant establishment (Pedrini *et al.* 2017). Coating mixtures of native species may also improve agroecosystem resilience and stability by increasing functional diversity (Madsen *et al*. 2012).

Although seed coating technologies have been primarily developed for major agricultural species (Pedrini *et al*. 2017), there is limited literature regarding suitable coating material and operational parameters for small native grasses. In addition, many studies on seed coating lack robust experimental design (Rajeshwari *et al*. 2020). Statistical experimental design and response surface methodology provide powerful tools to analyze factor interactions, reduce experimental effort, and model complex biological responses (Pedrozo *et al*. 2024; Leardi, 2009).

Here, we hypothesized that the coating process would not negatively affect the germination of *L. crinita*, *P. caespitosum*, and *D. californica*. To test this hypothesis, the present study optimized key factors in the mechanical coating of small amaranth seeds a model organism due to its similar morphology (Zubillaga *et al*. 2024). The optimized conditions were then validated with the target native grasses by evaluating pellet size, seed conglomeration, and germination performance.

This study provides an original integrative approach, combining statistical optimization with the use of a model organism. Focusing on seed-level responses, the research explores variability and highlights how pellet composition and seed number influence germination and early seedling development.

Materials and Methods

Plant Material

Because obtaining spikelet-free grass seeds is a labor-intensive process and a consistent seed supply was required for process optimization, commercial amaranth seeds were used as a model due to their morphological similarity to native grasses (Xavier *et al*. 2019).

For validation, seeds of *L. crinita*, *P. caespitosum*, and *D. californica* (*Poaceae*) were provided by the native species germplasm bank (ARG1416) at the Plant Resources Cabinet from Universidad Nacional de San Juan (UNSJ). Seeds were mechanically scarified and air-cleaned to obtain naked caryopses.

Seed Coating Optimization Procedure and Factors

Lavanya *et al*. (2011) described for mechanisms of pellet formation: nucleation, coalescence, layering, and abrasion. In the present study, nucleation was excluded because the seed served as the nucleus. Coalescence refers to the formation of larger particles from multiple nuclei; layering involves the successive addition of powder layers; and abrasion encompasses the material exchange between forming particles.

Based on previous reports and acknowledging that the sequential addition of filler and binder significantly affects final pellet size (Rajeshwari *et al*. 2020), the process was structured into 16 coating stages (table 1).

Table 1. Seed coating stages.

Tabla 1. Etapas del recubrimiento de semillas.

Stage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Binder	X	X	X	X	X	X	X	X	X	X	X	-	X	-	X	-
CO_3Ca	X	-	X	X	X	X	X	-	X	X	X	X	X	X	-	-
Clay	-	X	-	-	-	-	-	X	-	-	-	-	-	-	X	X

Quantitative and qualitative properties of the coating materials (table 2, page 4) were selected following ecological and sustainability criteria, key principles for ecological restoration (Hoose *et al*. 2019).

Table 2. Quantitative and qualitative factors considered.
Tabla 2. Factores cuantitativos y cualitativos considerados.

Factors related	References for factor and level consideration	Qualitative	Quantitative	Quantity	Unit
Seed	Rotary coater	Fixed	Fixed	30	g/proc.
Filler	Kangsopa *et al.* 2018	Fixed	Fixed	8.8/4.2	g/ stage
Binder	Afzal *et al.* 2020	Fixed	Variable	1.6 - 2.4	mL
Colorant	Afzal *et al.* 2020	Fixed	Fixed	1	g
Process	Working equipment		Fixed	16	stages
Pressure	Rotary coater		Fixed	1	atm
Temperature	Rotary coater		Fixed	25	°C
Plate speed	Pasha *et al.* 2016		Variable	490 - 630	rpm
Atomizer disc speed	Pasha *et al.* 2016		Fixed	420	rpm
Stage time	Just *et al.* 2013		Variable	30 - 40	sec/ stage

* Factors were considered under ecological restrictions
* Los factores se evaluaron dentro de restricciones ecológicas.

Filler: A 70:30 (w/w) mixture of calcium carbonate and diatomaceous earth (Kangsopa *et al*. 2018). White clay (kaolin) was used as a surface-smoothing agent (Romo-Campos & Guzmán Valle, 2019). Binder: Gum arabic (25% w/v), a natural water-soluble polysaccharide with a viscosity of 156.7 cP at 25°C, was used. Colorant: A food-grade powder dye was added for visual differentiation. After reviewing literature, it was decided to optimize three independent variables: plate rotational speed (490, 560, and 630 rpm), binder volume (1.6, 2, and 2.4 mL), and stage time (30, 35, and 40 s).

Evaluated Responses on the Optimization and Validation

Thirty pellets were randomly selected for measurement. Pellet size was determined by recording the major and minor diameters (mm) using a digital caliper. Size heterogeneity was calculated as the standard deviation of these diameters. To determine the number of seeds per pellet, each pellet was gently crushed, and the enclosed seeds were counted under a stereomicroscope. The amount of residue was quantified by collecting and weighing (g) the material adhered to the drum and plate of the rotary coater after coating. This value was subtracted from the total input weight of filler and binder to estimate the proportion of material loss.

Numerical Optimization of Multiple Responses

Using the fitted models, a numerical multi-response optimization was performed. The objectives were to: maximize pellet size, minimize the number of seeds per pellet (target = 1), minimize size heterogeneity, and minimize residue amount.

Pellet Validation

A single optimal point derived from the numerical optimization was selected to validate the desirability function and predictive models. For this purpose, a mixture of 30 g of cleaned caryopses was prepared, comprising 10 g each of *L. crinita*, *P. caespitosum*, and *D. californica*.

Additional Responses Evaluated during Validation

Dissolution: Pellets were placed on germination paper moistened and saturated with distilled water. The time required for complete disintegration was recorded.

Fragmentation: Pellets were placed in a 15 mL Falcon tube and subjected to continuous vibration for 1 min using a vortex shaker (Arcano) at 800 rpm. The number of broken pellets was counted (modified from Rajeshwari *et al*. 2020).

Germination Responses

To evaluate the effect of coating on germination, 30 pellets were sown in sterile Petri dishes lined with germination paper moistened with 6 mL of distilled water. Treatments included (four replicates each): caryopses (control), consisting of 10 seeds from each native species; single-seed pellets, diameter < 1.6 mm; and multi-seed pellets (conglomerates), diameter between 1.6 and 2.8 mm, containing an average of five seeds per pellet (150 seeds total) (Hoose *et al*. 2019). Plates were incubated in a germination chamber at 25°C under a 12 h light/dark cycle for five days. Germination was recorded when radicle emergence was visible, and shoot and radicle lengths were measured with a caliper.

Equipment

The coating process was performed using a rotary coater (SATEC Concept model ML 2000, SATEC Equipment GmbH, Germany (figure 1).

1- liquid pipeline, 2- baffles, 3- rotary coater plate, 4- atomizer disc.

1- Conducto para líquido. 2- Deflectores. 3- Plato giratorio recubridor. 4- Disco atomizador.

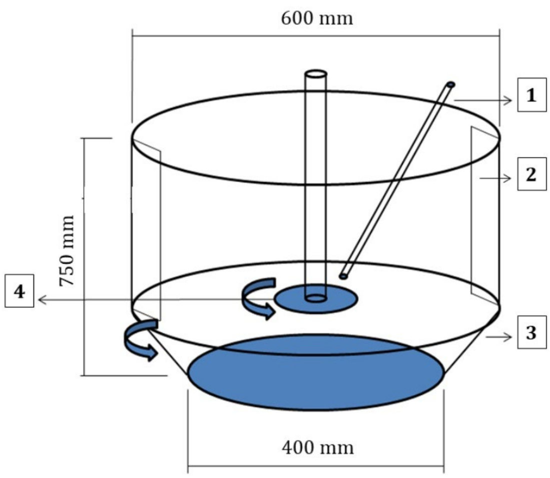


Figure 1. Schematic representation of the rotary coater (SATEC Concept model ML 2000).
Figura 1. Esquema interno. Modelo Concept SATEC ML 2000.

Statistical Analysis

All experimental design (Box-Behnken) and analyses were performed using Design-Expert 7.0.0 (Stat-Ease Inc., USA). Model significance, lack of fit, adequacy, precision, robustness, and numerical optimization were assessed. ANOVA assumptions, outliers, and data transformations were also analyzed to ensure model validity.

Results

Seed Coating Optimization

Table 3 summarizes the factor level used in the Box–Behnken experimental design and the corresponding responses obtained from the coating process for each experimental run.

Table 3. Box–Behnken design showing variation in plate speed, stage duration, and binder volume, and their corresponding responses: pellet size, seed number, residue amount, and size heterogeneity.

Tabla 3. Diseño Box-Behnken: Variación de factores: velocidad del plato, tiempo por etapa y cantidad de aglutinante; y resultados de la optimización: tamaño del pellet, cantidad de semillas, cantidad de residuo y heterogeneidad del tamaño.

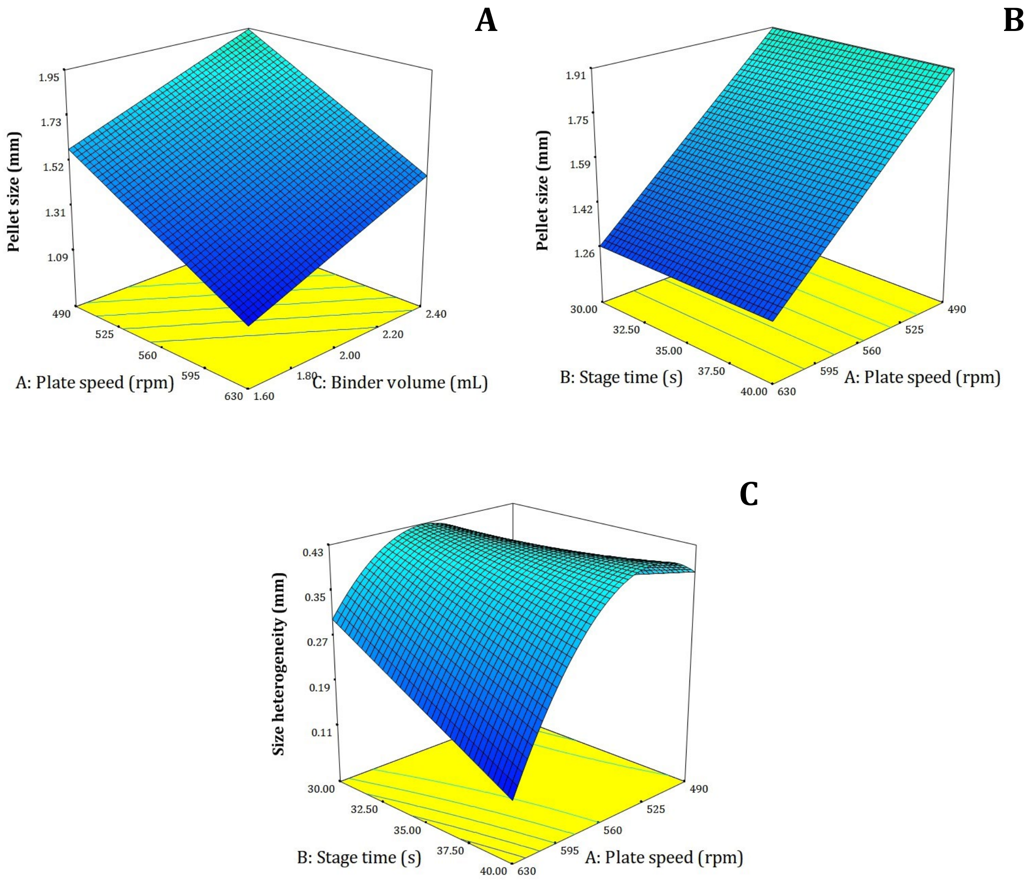
	Factors			Responses			
Run	Plate rotational speed (rpm)	Stage time (s)	Binder amount (mL)	Pellet size (mm)	Number of seeds (*posteriori* rounded)	Residue amount (g)	Size heterogeneity standard deviation (mm)
1*	560	35	2	1.58	0.9	64.89	0.35
2*	630	30	2	1.28	0.8	42.98	0.29
3	630	35	2.4	3.99	6.9	0	0.90
4	560	35	2	1.9	2.8	63.89	1.26
5	490	35	2.4	2.37	4	80.9	0.55
6*	560	35	2	1.74	1.2	24.02	0.44
7*	630	40	2	1.07	0.9	71.12	0.11
8	560	40	1.6	1.85	2.3	63.74	0.63
9*	490	30	2	1.68	1.1	95.46	0.27
10*	560	30	2.4	1.83	0.8	130.52	0.27
11*	560	30	1.6	1.32	1.2	79.82	0.60
12*	490	40	2	1.5	1	83.75	0.38
13*	490	35	1.6	1.73	1.3	77.36	1.01
14	560	40	2.4	1.57	1.6	87.56	0.60
15*	630	35	1.6	1.09	0.9	87.68	0.33

* Runs for single-seed pellet size model.

* Corridas para el modelo de tamaño de pellets de una semilla.

Modeling of Responses

Pellet size data were analyzed in two subsets: (A) single-seed pellets (figure 2A, page 4) and (B) all data combined (figure 2B, page 7), including multi-seed (conglomerate) pellets (Hoose *et al.* 2019). All response models were significant (table 4, page 7) and showed no lack of fit unless otherwise indicated. Adjusted and predicted R^2 values were consistent, and ANOVA assumptions were met.



(A) Single-seed pellets; (B) combined dataset (single- and multi-seed pellets); (C) size heterogeneity (standard deviation) of single-seed pellets. Binder volume fixed at 2 mL.

A- Tamaño del pellet con una sola semilla. B- Todos los datos, tamaños de pellets con una y múltiples semillas. C- Heterogeneidad del tamaño en pellets de una sola semilla (desviación estándar). Volumen de aglutinante fijo en 2 mL.

Figure 2. Influence of the evaluated factors on pellet size.
Figura 2. Influencia de los factores evaluados sobre el tamaño del pellet.

Table 4. Responses and models/ Statistical summary of the fitted models.
Tabla 4. Respuestas y modelos/ Resumen estadístico de los modelos ajustados.

Response	Adj. R^2	Model
(A) Pellet size: Single-seed pellets	0.61	Pellet size = 2.57 - 3.5*10 $^{-3}$ * P_S + 0.45 * B_V
(B) Pellet size: All data	0.42	Pellet Size = 4.16 - 4.60*10^{-3} * P_S
Size heterogeneity: Single-seed pellets	0.96	Size Heterogeneity = - 6.88 + 0.02 * P_S + 0.35 * S_T - 2.43 * B_V - 2.08*10 $^{-4}$ * P_S * S_T + 9.99*10^{-3} * P_S * B_V - 0.11 * S_T * B_V - 2.78*10^{-5} * P_S ^{2}
Size heterogeneity: All data		No Fit
Number of seeds Bartlett, M. S. (1947).	0.36	Sqrt (Number of seeds) = - 0.26 + 0.76 * B_V
Residue amount	0.63	Residue amount = - 1704.79 + 3.13 * P_S + 960.47 * B_V - 1.69 * P_S * B_V

* (P_S: Plate speed, B_V: Binder volume, S_T: Stage time)

* (P_S: Velocidad del plato, B_V: Volumen del aglutinante, S_T: Tiempo de etapa).

Pellet Size and Size Heterogeneity

Models indicated that increasing the rotational speed of the rotary coater plate reduced pellet size (figure 2A-B, page 7). Conversely, greater binder volume increased pellet size for single-seed pellets (figure 2A, page 7).

Size heterogeneity (figure 2C, page 7) was analyzed only for single-seed pellets, since combining single-and multi-seed data was better represented by the overall mean (mean = 0.54). Longer stage duration and higher rotation speed reduced size variability among pellets.

Number of Seeds per Pellet

The model describing the number of seeds per pellet was significant (adjusted R^2 = 0.36; figure 3). Although predictive precision was moderate, the model adequately represented overall behavior (Pedrozo *et al*. 2024). Increasing binder volume led to the formation of conglomerates.

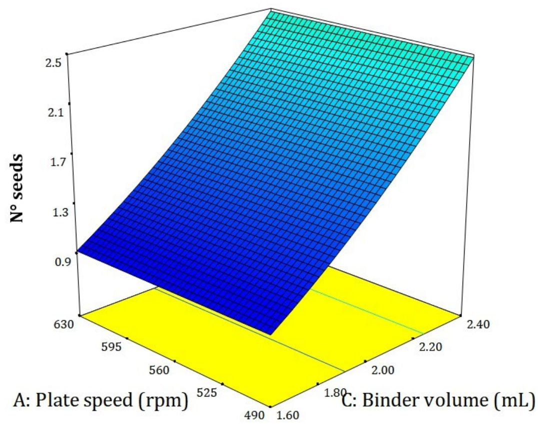


Figure 3. Influence of the evaluated factors on the number of seeds per pellet.

Figura 3. Influencia de los factores evaluados sobre la respuesta de cantidad de semillas.

Amount of Residue

During coating, some material adhered to the rotary drum and plate, contributing to residue accumulation (figure 4).

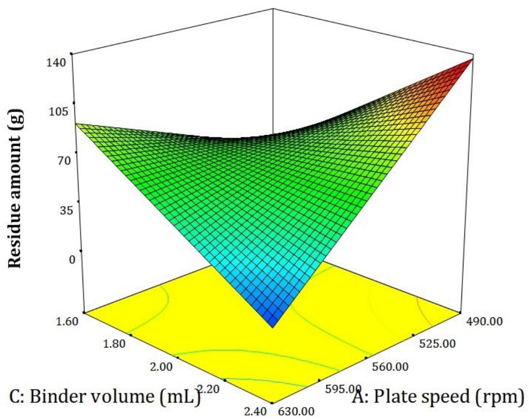


Figure 4. Effect of evaluated factors on the residue adhered to the rotary coater drum and plate at the end of process.

Figura 4. Influencia de los factores evaluados sobre la cantidad de residuo adherido al tambor y al plato de la peletizadora al final del proceso.

The smallest amounts of residue occurred at high plate rotation speed and maximum binder volumes, which coincided with the formation of conglomerates. Conversely, low plate speeds combined with high binder volumes produced the largest residues.

Residue per experiment ranged from 0 to 140 g, averaging 43% of total input mass.

Multi-Response Optimization

A simultaneous optimization was performed using desirability criteria for the following responses: pellet size (maximize, importance: +++), size heterogeneity (minimize, +), number of seeds (minimize, ++++), and amount of residue (minimize, ++). The optimal compromise solution is shown in figure 5. All models used corresponded to the complete dataset (single- and multi-seed pellets).

Pellet size (maximize +++), size heterogeneity(minimize+), amount of seed (minimize +++++), and amount of residue (minimize ++).

Tamaño del pellet (maximizar +++), Heterogeneidad del tamaño (minimizar +), Cantidad de semillas (minimizar +++++), y Cantidad de residuo (minimizar ++).

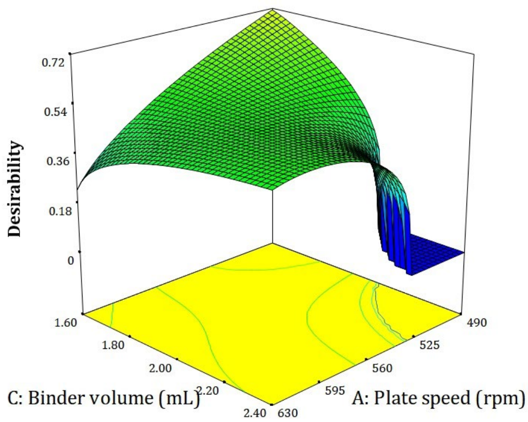


Figure 5. Multiple-response optimization based on experimenter-defined criteria.

Figura 5. Optimización de múltiples respuestas según criterios del experimentador.

Maximum desirability was achieved at low plate speed and low binder volume. A marked decrease in desirability occurred under conditions of low plate speed and high binder volumes, corresponding to zones of formation of conglomerates and high residue accumulation.

Prediction and Validation

Model predictions were validated using the following optimized conditions: plate speed = 595 rpm, binder volume = 2.2 mL, and stage duration = 35 s (overall desirability: 0.51). Validation used caryopses of *L. crinita*, *P. caespitosum*, and *D. californica* (table 5, page 10).

Pellets were classified into four size categories, and validation results were calculated as weighted means based on proportional mass. Two additional responses were also evaluated: fragmentation and dissolution time.

Weighted means: pellet size = 1.38 mm; number of seeds per pellet = 3.15; heterogeneity (SD) = 0.36. Single-response value: amount of residue = 25% at the end of the process.

Predicted values for these conditions are presented in table 6 (page 10).

All validation results fell within the 95% confidence intervals predicted by the models.

Germination and Seedling Performance

Pellets obtained during validation (table 6, page 10) were used to evaluate germination and early seedling development in native grasses (*L. crinita*, *P. caespitosum*, and *D. californica)*.

Germination

No significant differences were detected in germination percentage between control caryopses and single-seed pellets (figure 6, page 10). However, multi-seed (conglomerate) pellets showed a 20% reduction compared to both control and single-seed treatments.

Table 5. Validation responses: Pellet size, size heterogeneity, and number of seeds after classification into four size categories, expressed as weight percentage of the total mix. Additional responses: Fragmentation and dissolution time.

Tabla 5. Respuestas de validación: Tamaño del pellet, heterogeneidad del tamaño y número de semillas tras el tamizado en cuatro tamaños, y porcentaje de representación del total de la mezcla en peso. Respuestas adicionales: Fragmentación y disolución.

Size category	Size average (mm)	Size heterogeneity (standard deviation size)	Number of seeds (mean)	Percentage representation (% by weight)	Fragm. (%)	Dissol. (s)
Fine	1.1	0.12	1	34	50	< 3
Medium	1.2	0.09	1.4	43	60	< 3
Coarse	1.6	0.62	5.4	18	30	< 3
Very coarse	2.85	0.58	9.2	5	0	< 3

* Fragm.: violent fragmentation; Dissol.: pellet dissolution time.

* Fragm.: Fragmentación violenta, Dissol.: Tiempo de disolución del pellet.

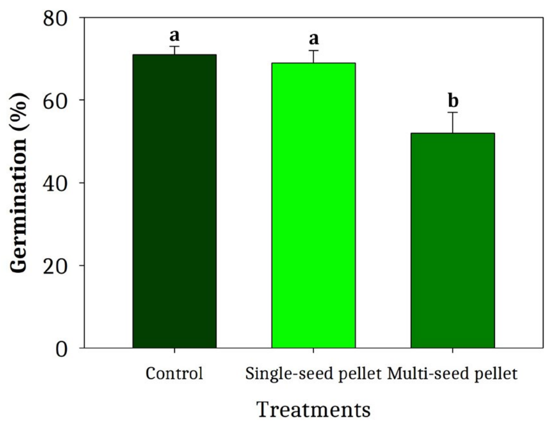
Table 6. Model-predicted responses under optimal conditions (plate speed 595 rpm; binder volume 2.2 mL; stage time 35 s).

Tabla 6. Predicción de las respuestas del pellet bajo las condiciones: velocidad del plato (595 rpm), volumen de aglutinante (2,2 mL), tiempo por etapa (35 segundos).

Response	Prediction	95% CI low	95% CI high	Validation (weighted mean)
Seed size	1.42	1.22	1.62	1.38
Seed quantity	2.22	1.37	3.28	3.15
Heterogeneity	0.54	0.36	0.71	0.36
Amount of residue	43.21	21.15	65.26	25*

* Single response, not weighted mean.

* Respuesta individual, no promedio ponderado.



Bars indicate: standard deviation; different letters denote significant differences (Fisher's LSD, α < 0.05).

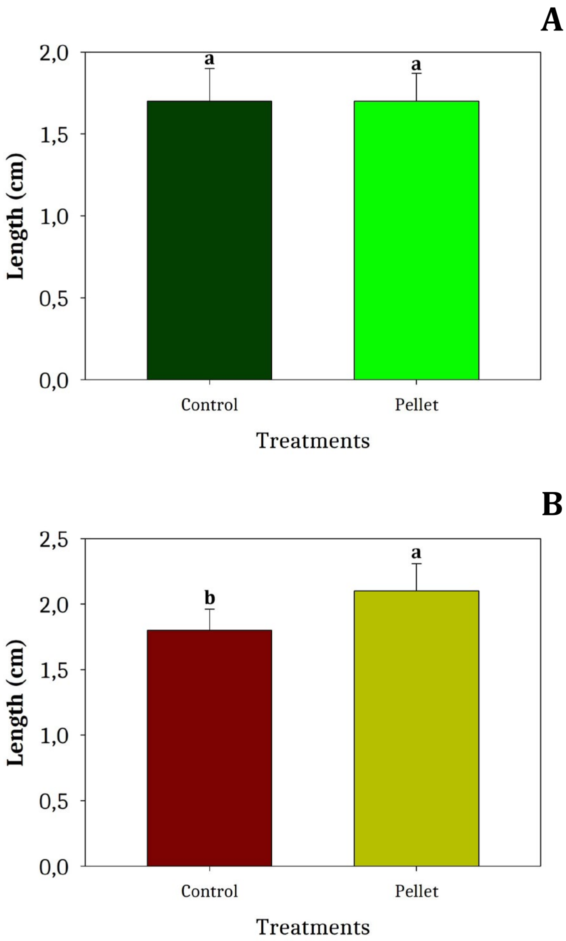
Las barras indican la desviación estándar. Letras diferentes indican diferencias significativas (LSD de Fisher, α < 0,05).

Figure 6. Germination percentages for caryopses (control), single-seed pellets, and multi-seed pellets. Species: *L. crinita*, *P. caespitosum*, and *D. californica*.

Figura 6. Porcentajes de germinación obtenidos en los tratamientos de cariopses (control), pellets de una semilla y pellets de múltiples semillas. Especies: *L. crinita*, *P. caespitosum* y *D. californica*.

Shoot and Radicle Length

Shoot and radicle length results are shown in figure 7. Shoot length did not differ significantly among treatments. In contrast, radicle length was significantly greater in pelleted seeds (2.12 ± 0.17 cm) than in controls (1.78 ± 0.11 cm).



Bars represent standard deviation; different lowercase letters indicate significant differences (Fisher's LSD, α < 0.05).

Las barras indican la desviación estándar. Letras minúsculas diferentes indican diferencias significativas (LSD de Fisher, α < 0,05).

Figure 7. (A) Shoot and (B) radicle length in caryopses (control) and pellet treatments for *L. crinita*, *P. caespitosum*, and *D. californica*.

Figura 7. (A) Longitud de plántula y (B) longitud de radícula para los tratamientos: cariopses (control) y pellets. Especies: *L. crinita*, *P. caespitosum* y *D. californica*.

Discussion

This study provides one of the first comprehensive descriptions of the seed-coating process for small-seeded species, detailing component quantities, proportions, processing times, and operational stages. It also represents the first statistical optimization of seed coating for native grass from arid regions. Although seed-coating technologies have been developed for decades, detailed methodological information is rarely available in the public domain, as most advances are confined to private industry (Pedrini *et al*. 2017).

Optimization of Individual Responses

The fitted models showed that the rotational speed of the coater plate had a significant influence on pellet size and homogeneity. The shear effect associated with higher speeds likely caused a reduction in pellet size (figures 2A-B, page 7) due to material detachment from the forming pellet (Iparraguirre, 2009; Pasha *et al*. 2016). Conversely, increasing binder volume in single-seed pellets resulted in larger pellet (figure 2A, page 7), in agreement with reports from the pharmaceutical industry where higher impeller speeds reduce pellet size, whereas binder concentration increases it (Thies & Kleinebudde, 1999). Longer stage durations were associated with reduced size heterogeneity (figure 2C, page 7), in line with the findings of Just *et al*. (2013). The combination of layering and abrasion appears to benefit from extended processing times, promoting more uniform pellets. Heterogeneity also decreased with increasing plate speed, confirming the observations made by Pasha *et al*. (2016). Regarding the number of seeds per pellet (figure 3, page 8), higher binder viscosity induced by increased binder volume promoted seed adhesion and coalescence (Johansen & Shaefer, 2001; Keningley *et al*. 1997; Lavanya *et al*. 2011). For residue response (figure 4, page 8), a relevant factor considering the effort required to obtain spikelet-free native grass seeds (Meglioli *et al*. 2024), the combination of greater conglomerate mass and higher rotational speed enhanced the detachment of adhering material, reducing residue (Iparraguirre, 2009). In contrast, low speed combined with high binder volume increased adhesion to the plate and drum, resulting in higher residue levels (Johansen & Shaefer, 2001). The mean residue of 43% per run was lower than that reported by Pedrini *et al*. (2017) for *Solanum lycopersicum* (65.3%) and *Microlaena stipoides* (60.9%).

Multi-Response Optimization, Validation, and Germination in Native Seeds

Multi-response desirability analysis successfully identified optimal coating conditions. Validation confirmed both the predictive accuracy and robustness of the statistical models, as all measured values for native grass seeds fell within the 95% confidence intervals of model predictions.

Seed coating can enhance or impair seed physiology by facilitating water and nutrient uptake or, conversely, by impeding radicle emergence (Pedrini *et al*. 2017; Sprey *et al*. 2019). In the present study, germination of single-seed pellets was unaffected, in agreement with the results obtained using comparable materials (Romo-Campos & Guzmán Valle, 2019). In contrast, multi-seed pellets (conglomerates) showed reduced germination, possibly due to allelopathic interactions among seeds during germination, as reported in other grass species (Möhler *et al*. 2018). The enhanced radicle length observed in pelleted seeds may be associated with the coating components ($CaCO_3$, diatomaceous earth, kaolin), which provide calcium and silicon, elements linked to cell elongation, improved nutrient transport, and stress tolerance (Bauer *et al*. 2011; Jalal *et al*. 2023). Additionally, the high intraspecific genetic variability of native grasses (Trod *et al*. 2018; Villagra *et al*. 2011), combined with the mixed caryopses from three species, could explain the variability seen in physiological responses.

Conclusions

Validation using small-seeded native grasses confirmed the applicability, accuracy, and robustness of the statistical optimization methodology. All pellet traits fell within the predicted confidence intervals, supporting the reliability of the models. Seedling evaluations indicated that coating did not negatively affect germination, possibly due to the rapid dissolution of the coating material, and that the process positively influenced radicle elongation.

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.